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14. ABSTRACT

The Persian Gulf War resulted in friendly fire casualties among U.S. personnel injured by fragments of depleted uranium (DU) munitions. The demonstrated effectiveness of such weapons makes it likely that they may be used against U.S. forces in future conflicts. Uncertainty about how aggressively to remove fragments of the radioactive, chemically toxic DU has stimulated research into the long-term health consequences of embedded DU fragments. There has been no previous research to determine whether long-term exposure to embedded DU can affect the health of offspring of personnel wounded by DU. This study investigated whether male mice carrying embedded fragments of DU or WA transmitted genetic damage to their offspring. We hypothesized that long-term chronic exposure to embedded DU or WA would result in paternal transmission of genetic damage to unexposed F1 generation offspring, characterized by increased frequency of in vivo mutations in tissues. The data demonstrated that DU and WA can induce genomic instability in unexposed offspring. The findings also show that DU and WA are mutagenic to chronically exposed rodents. DU and WA can cause direct DNA damage to sperm in these chronically exposed rodents.

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Introduction

Several potentially toxic heavy metals have been used on the battlefield. These metals included both depleted uranium (DU) and tungsten alloys (WA). The Persian Gulf War in 1991 saw the first combat use of DU kinetic penetrator munitions, and their success against enemy armor was dramatic. This use led to a friendly fire incident which resulted in the wounding of U.S. military personnel by DU shrapnel. The demonstrated effectiveness of DU munitions in the first Gulf War has led other nations, some not friendly to the United States, to adopt these weapons into their own arsenals. Other types of kinetic energy penetrators use heavy-metal tungsten alloys (WA) in place of DU. In future conflicts, the United States will have to deal with an increased number of casualties from the use of these weapons. Because both DU- and WA-based munitions are relatively recent additions to the list of militarily relevant metals, little is known about the long-term health effects of these metals after internalization as embedded shrapnel. It is unknown as to whether long-term exposure to embedded DU- or WA- fragments could potentially affect the health of an offspring of a soldier wounded with these metals is unknown. Preconceptional paternal exposure (PPE) to either radiation or other metals such as lead, have been implicated in the etiology of multiple childhood cancers This study was designed to investigate the paternal transmission of genetic damage in offspring of male rodents carrying embedded depleted uranium fragments using a transgenic mouse model. This system allows us to assess *lac*I gene mutation frequency in tissues obtained from progeny. The specific aims of the project included: 1) Determine whether chronic preconceptional paternal exposure to DU or WA causes a paternal transmission of genetic damage to F1 progeny; 2) Measure the mutagenic frequency in reproductive organs of DU- or WA- implanted male mice chronically exposed and 3) Assess potential direct damage to paternal spermatozoa DNA. This research addresses the DOD effort to understand the potential health risks associated with DU and WA exposure in order to develop appropriate medical treatment protocols and guidance for personnel wounded by fragments of these metals.

Body

Statement of Work:

The objective of this study is to investigate the paternal transmission of genetic damage in offspring of male rodents carrying embedded depleted uranium fragments using a transgenic mouse model which allows us to assess *lac*I gene mutation frequency in bone marrow tissues obtained from F1 progeny; using this model we can examine tissue mutation frequency in chronically-exposed animals and their F1 generation offspring. This mutation system employs a λlacI shuttle vector carried by cells of a transgenic mouse (Big Blue: strain C57BL/6 hemizygous mice containing 40 copies of a λlacI shuttle vector per cell). Big Blue male mice will be implanted with DU or WA pellets and mated to unimplanted female mice. To assess mutagenesis, DNA (λlacI shuttle vector) will be recovered from specific organs. The packaged λlacI shuttle vector is then assayed in vitro for mutations, detected in viral plaques. Responses in these F1 generation mice will be compared to progeny from 1) mice implanted with the inert metal tantalum 2) mice implanted with the heavy metal alloy (WA), and 3) mice irradiated whole-body with low-dose neutron (high LET) radiation. In implanted males/fathers we will assess tissue metal content and in vivo mutagenesis in kidney, liver, and bone marrow at selected times after implantation. The bone marrow of F1 offspring will be assessed for mutagenicity. DNA damage in spermatozoa of metal-implanted mice will be assessed by the DNA damage assay commonly known as the "comet assay". We will assess the effects of two doses (2 or 6 pellets) of DU and HMTA and a single dose (6 pellets) of Ta. For comparison, a single low-dose of neutrons will be used (2 Gy) Subgroups of animals will be similarly implanted/irradiated but euthanized at 1, 4, and 7 months to allow for testing of kidney metal content, sperm DNA damage, and tissue mutagenicity.

Progress to Date

This is the final report for this project. All three aims/tasks associated with the project have been successfully completed. Research results for each aim/task are summarized below.

Aim/Task 1: Determine paternal transmission of genetic damage to unexposed F1 generation offspring. Determine whether preconceptional paternal exposure causes a paternal transmission of genetic damage to F1 progeny characterized by an increased frequency of LacI mutations in offspring bone marrow in comparison to progeny of un-implanted fathers. The

mutagenicity will be assessed using the Big Blue transgenic mouse system which allows mutations induced in vivo to be analyzed in vitro.

Methods

The mutagenicity was assessed using the Big Blue transgenic mouse system which allows mutations induced *in vivo* to be analyzed *in vitro*. The Big Blue mutation system employs a λ shuttle vector carried by cells of a transgenic mouse (Stratagene "Big Blue", C57Bl mouse). Big Blue mice were implanted with DU or WA pellets for 1, 4, and 7 months, and then mated with unimplanted nontransgenic females (C57Bl). Multiple litters per implanted male were obtained. Litter number at birth was noted. Pups were considered adults at weaning (12 weeks old). Surviving pup numbers at weaning were observed as well since in some cases not all live-at-birth pups survived to weaning. To assess for offspring genotype for the LacI gene, genotyping analysis was conducted using polymerase chain reaction (PCR) analysis. Pups carrying the LacI gene were used for analysis of genetic damage. Pups that did not carry the gene were euthanized. DNA was recovered from bone marrow tissues of the F1 progeny that carried the LacI gene. Packaged λ shuttle vector was assayed *in vitro* for mutations, detected in viral plaques by the blue color resulting from metabolism of X-gal. A mutagenic response or enhancement is manifested by an increased frequency of in vivo mutation in comparison to progeny of un-implanted fathers. The data are reported as a mutation frequency in offspring bone marrow assessed in the F1 progeny. The mutation frequencies in F1 progeny of DU-implanted fathers will be compared to those of F1 progeny from WA-implanted, Tantalum (Ta)-implanted and neutron-irradiated fathers. Two doses of DU or WA pellets was be used in our experiments (low dose-2 pellets, high dose-6 pellets). Thus in the current study we had a low and high dose of DU. All implanted male mice received 6 pellets. For example, 2 DU plus 4 Ta or 6 DU plus no Ta pellets. Ta, an inert metal used in prosthetic devices was used as a negative surgical control. Neutron radiation (2 Gy) was be used for comparison as a high LET radiation comparison.

Results

Litter Characteristics: Transgenic male mice were implanted with DU or WA, or Ta. At 1, 4, or 7 months post-metal implant, the males were mated with unimplanted female mice. After the litters were born, offspring pups were counted and observed for any specific-locus mutations. These are mutations such as baldness, anatomical abnormalities, etc. In the current study there was no statistical increase in these types of mutations. The visible specific-locus mutation assay requires a significantly increased number of offspring per group to provide statistical certainty.

Litter characteristics are shown in Tables 1 and 2. The number of offspring observed live at birth was noted for each group and is shown in Table 1. The number of offspring observed at the time of weaning was also noted and is shown in table 2. The data indicate that one month post DU or WA implantation, only the litters from DU-implanted fathers demonstrated a decrease in litter number in comparison to un-implanted controls. At four months post metal implantation, litters from both DU- and WA- implanted fathers (low and high dose) showed a significant decrease in average litter number in comparison to controls. In contrast at seven months post pellet implantation, average litter number for offspring from WA implanted fathers did not show a significant change from controls. The average litter size from DU-implanted fathers (seven months) decreased to approximately 29% of seven month controls. Table 2 shows data from the average number of offspring alive at weaning (12 weeks old). In control animals the average litter number at the time of weaning is approximately 5 to 8% lower than at birth. This is characteristic of several strains of mice including C57Bl. The data in Table 2 demonstrate that there was no significant difference between the offspring from metal implanted mice and controls (at 1 and 4 months post-metal implantation) in terms of average litter size at weaning. In contrast, at seven months post-chronic exposure offspring from DU (high dose) and WA (low and high dose) showed a 28 to 23% reduction in survival at weaning in comparison to the number of offspring survival at birth. Data show that although offspring from WA –implanted fathers exhibited a decrease in survival at weaning they demonstrated a greater survival rate at birth.

Offspring genotyping: The transgene was identified in the F1 offspring by using PCR analysis. The results from the genotyping are shown in Table 3. The data show that compared to the expected 50% transmittal rate, the observed rate for the offspring from control and DU/WA-metal-implanted fathers, does not significantly differ ($P > 0.05$). Only the F1 offspring that carry the lacI gene were assessed for lacI mutations.

Mutation Frequencies in F1 Offspring: The mean mutation frequencies in F1 offspring bone marrow tissue are summarized in Table 4. Bone marrow was obtained from LacI positive offspring at 14 to 16 weeks post birth for all groups. Mutation frequencies in shuttle vector recovered from bone marrow of F1 offspring mice from un-implanted fathers was not significantly different in each of the time periods tested. Mutation frequencies in offspring from DU implanted fathers (4 and 7 months post DU implantation) showed increased mutation frequency in contrast to control offspring. The data show that offspring from fathers exposed to DU for one month did not exhibit a change in mutation frequency. Similarly, F1 offspring from WA-implanted fathers (high dose) exhibited an increased mutation frequency at 4 and 7 months

post pellet implantation. Offspring from fathers exposed to low-dose WA did not exhibit a significant increase at 4 months post-implantation. In comparison studies, males mated at 30 days post neutron radiation exposure did not produce any offspring therefore we mated the irradiated males at 65-70 days post radiation exposure. The lack of offspring at 30 days post whole body neutron radiation has been shown previously. Offspring from neutron-irradiated fathers (2 Gy, + 68 days) demonstrated a significant increase in bone marrow mutation frequency as seen in Table 4. Offspring from tantalum-implanted fathers did not show any significant difference in bone marrow mutation frequency in comparison to control offspring.

Aim/Task 2: Measure mutagenic potential of embedded fragments of DU and WA relative to neutron radiation, and the biologically inert metal tantalum, in testes of implanted male mice and correlation with tissue-metal content Determine the mutagenic frequency in implanted male mice chronically exposed to DU, WA, or TA (1, 4, 7 months). The mutagenicity will be assessed using the Big Blue transgenic mouse system which allows mutations induced *in vivo* to be analyzed *in vitro.*

Methods

The mutagenicity was assessed using the Big Blue transgenic mouse system which allows mutations induced *in vivo* to be analyzed *in vitro*. The Big Blue mutation system employs a λ shuttle vector carried by cells of a transgenic mouse (Stratagene "Big Blue", C57Bl mouse). Big Blue mice were implanted with DU or WA pellets for 1, 4, and 7 months. At 1, 4, or 7, month's post-metal implantation, males were euthanized and tissues were collected for analysis. Packaged λ shuttle vector was assayed *in vitro* for mutations, detected in viral plaques by the blue color resulting from metabolism of X-gal. The data are reported as a mutation frequency in testes, kidney, and liver obtained from implanted males. Two doses of DU or WA pellets was be used in our experiments (low dose-2 pellets, high dose-6 pellets). Thus in the current study we had a low and high dose of DU. All implanted male mice received 6 pellets. For example, 2 DU plus 4 Ta or 6 DU plus no Ta pellets. Ta, an inert metal used in prosthetic devices was used as a negative surgical control. Neutron radiation (2 Gy) was be used for comparison as a high LET radiation comparison.

Results

The mutation frequency in three tissues was assessed for implanted males. These tissues include the testes, kidney, and liver. The findings are presented in Table 5. Males implanted with DU

exhibited a dose- and time- dependent increase in mutation frequency in testes tissues. Even at one month post DU or WA implantation, the testes demonstrated an increase in mutation frequency. At the highest DU and WA doses (7 months exposure) there was a 70.1 and 50.2- fold increase in mutagenicity. Similarly WA-implanted males showed a dose- and time- dependent increase in mutation frequency to a similar degree. Tantalum-implanted males did not demonstrate any significant change in mutation frequency in testes tissues in comparison to controls. Data obtained with kidney and liver tissue also demonstrated that DU and WA chronic exposure resulted in a dose- and time- dependent increase in tissue mutagenicity. Similar time and dose dependent patterns were observed. These data are also shown in Table 5.

Levels of uranium, cobalt, nickel, tungsten, and tantalum were determined in testes tissues using inductively coupled plasma mass spectrometry (ICP-MS). These data are presented in the Appendices Figures 1, 2, 3, and 4. Data from DU implanted males demonstrate that there was a significant increase in testes uranium levels at 1, 4, and 7, months post pellet implantation. Similarly WA implanted males exhibited a significant increase in tungsten. Nickel, cobalt in the tissues tested.

Aim/Task 3: Measure DNA damage in paternal spermatozoa after chronic DU or WA exposure. Measure the direct or targeted damage to the paternal spermatozoa DNA using the single cell gel electrophoresis "Comet" assay. The intent of this task was to measure the direct or targeted damage to the paternal spermatozoa DNA using the single cell gel electrophoresis "Comet" assay. Mouse spermatozoa will be collected from the vas deferens at post-mortem and processed for the comet assay. Single cell gel electrophoresis will allow us to use a new methodology to effectively assess direct sperm DNA damage. In contrast to convention methodology and cell lysis protocols, this methodology will enable us to remove the tightly bound protamine molecules from the spermatozoa DNA. This DNA damage analysis in combination with the F1 progeny mutation frequencies will allow us to speculate as to the mechanism of the potential transgenerational transmission of genetic damage. DNA damage measurements will assess germ-cell mutations and F1 mutation frequencies will measure whether genetic damage induced in a germ cell leads to the appearance of mutations in succeeding cell generations and /or in embryonic cell generations. Three parameters are measured Comet tail length, percentage of tail DNA, and tail moment. These parameters are measured using quantitative image analysis and Comet analysis software.

Results:

Chronic exposure of the testis in males implanted with DU produced dose – and time dependent increases in the level of DNA damage present in spermatozoa collected from the vas deferens (Table 6). At 1 month post metal implantation, there was no increase in sperm DNA damage in either DU or WA exposed males. However, statistically significant increases in Comet tail moment, tail length, and percentage of DNA were detected after exposure to high doses of DU or WA for 4 or 7 months). Increases in DNA damage were measured at lower doses of DU and WA at 4 and 7 months post metal exposure for both DU and WA. Specifically, increases in Comet tail moment range from 1.6 to 2.3 fold increase in DNA damage at 4 and 7 months exposure were observed. Similar changes were seen for percentages of tail DNA and tail moment. These data demonstrate that DU and WA cause a dose-dependent increase in sperm DNA damage.

Key Research Accomplishments

- Preconceptional Paternal DU exposure induced a decrease in offspring litter number at high and low doses at short exposure times
- Preconceptional Paternal WA exposure induced a significant decrease in litter survival
- Offspring from fathers Implanted with DU Exhibited Bone Marrow Mutagenicity
- Offspring from fathers implanted with WA exhibited bone marrow mutagenicity
- DU and WA induce genomic instability in unexposed offspring
- DU induced mutagenicity in male testes in a time- and dose- dependent manner
- WA induced mutagenicity in male testes in a time- and dose- dependent manner
- DU causes DNA damage in male sperm and testes
- WA causes DNA damage in male sperm at high doses
- DU and WA exposure did not induce solid tumors
- WA exposure induced splenomegaly.

Reportable Outcomes

Poster Presentation and Abstract - Miller, AC, R, Rivas, Merlot, R, Crepin L., Lison P. Preconceptional Paternal Exposure to Embedded DU and Tungsten Alloy Pellets in Vivo: Transmission of Genetic Damage and Transgenerational Effects. Preliminary Studies, USA Military Health Forum, April 2004, Puerto Rico

Oral Presentation and Abstract - Miller, AC, LeBlanc B, R, Rivas, Merlot, R, Crepin L., Lison P. Preconceptional Paternal Exposure to Embedded DU fragments: Transmission of Genetic Damage and Transgenerational Effects. IARC Symposium on Short-term Models of Carcinogenesis, Dec 2004, Paris, France

Oral Presentation and Abstract – Miller, AC. Preconceptional Paternal Exposure to Embedded DU fragments: Transmission of Genetic Damage and Transgenerational Effects. Uniformed Services University Health Sciences Research Days, May 2005.

Oral Presentation and Abstract - Miller AC, Beltran D, Rivas R, Stewart M, Merlot RJ, and Lison PB (2005) Radiation- and depleted uranium-induced carcinogenesis studies: Characterization of the carcinogenic process and development of medical countermeasures. Published in proceedings of the Human Factors and Medicine (HFM) Panel Research Task Group (RTG) 099 Meeting, "Radiation Bioeffects and Countermeasures," Bethesda, MD, USA, June 21-23, 2005. Compact disc: AFRRI CD 05-2

Oral Presentation and Abstract - McClain DE, Miller AC, and Kalinich JF (2005) Status of health concerns about military use of depleted uranium and surrogate metals in armor-penetrating munitions. Published in proceedings of the Human Factors and Medicine (HFM) Panel Research Task Group (RTG) 099 Meeting, "Radiation Bioeffects and Countermeasures," Bethesda, MD, USA, June 21-23, 2005. Compact disc: AFRRI CD 05-2.

Oral Presentation- Columbia University College of Physician and Surgeons, Feb 2005 Radiological Research Center, Seminar Series Oral Presentation: Preconceptional Paternal Exposure to Embedded DU fragments: Transmission of Genetic Damage

Poster Presentation and Abstract - Miller, AC, R, Rivas, Merlot, R, Crepin L., Lison P. Preconceptional Paternal Exposure to Embedded DU and Tungsten Alloy Pellets in Vivo: Transmission of Genetic Damage and Transgenerational Effects. American Association for Cancer Research, April 2006, Washington DC.

Poster Presentation and Abstract - Miller, AC, R, Rivas, Merlot, R, Crepin L., Lison P. Preconceptional Paternal Exposure to Embedded DU and Tungsten Alloy Pellets in Vivo: Transmission of Genetic Damage and Transgenerational Effects. USA Military Health Forum, May 2006, Puerto Rico

Oral Presentation and Abstract - Miller, AC. Development of Models to Study Radiation-Induced Late Effects. NATO Human Factors and Medicine Meeting, Umea, Sweden, June 2006.

Oral Presentation and Abstract - Miller, AC. Transmission of Genetic Damage and Transgenerational Effects. National Institute Occupational Safety and Health Metal Conference, Sept 2006, Morgantown, WV.

Manuscript:

Miller, AC. Radiation- and depleted uranium-induced carcinogenesis studies: Characterization of the carcinogenic process and development of medical countermeasures. Published in proceedings of the Human Factors and Medicine (HFM) Panel Research Task Group (RTG) 099 Meeting, "Radiation Bioeffects and Countermeasures," Bethesda, MD, USA, June 21-23, 2005. Compact disc: AFRRI CD 05-2

Personnel Employed

Rafael Rivas

Conclusions

This study was designed to investigate the paternal transmission of genetic damage in offspring of male rodents carrying embedded DU or WA fragments using a transgenic mouse model. This system allowed us to assess *lac*I gene mutation frequency in tissues obtained from chronically exposed fathers and unexposed offspring. The study also provided information regarding direct DNA damage in germ cells and reproductive organs of exposed males and whether DU or WA was mutagenic.

DU exposure induced an increase in offspring bone marrow mutagenicity in a dose- and time- dependent manner. WA exposure similarly induced offspring bone marrow mutagenicity at higher doses and at longer exposure times. Tantalum did not affect offspring bone marrow mutagenicity. Both chronic paternal DU or WA exposure caused a decrease in offspring litter size and survivability but paternal WA exposure had a more significant impact on the litter survivability of unexposed offspring.

Both DU and WA were mutagenic to exposed male reproductive tissues in a dose- and time- dependent manner and both heavy metals were able to induce significant sperm DNA damage that was also time- and dose- dependent.

To put these findings into perspective it is important to consider that the offspring did not exhibit any development of cancer and the induction of genomic instability in their bone marrow means that there is a mechanism for the development of cancer. Genomic instability has been implicated in childhood leukemia found in children on Chernobyl survivors but has not been observed in children of metal exposed individuals. Genomic instability has also been implicated in susceptibility to cancer development. In terms of the exposure of implanted males to DU or WA, the findings continue to support earlier studies demonstrating that both DU and WA are mutagens. These data are the first to demonstrate that DU or WA exposure can cause significant sperm DNA damage which may be involved in the observed transmission of the genetic damage to the unexposed offspring.

Critical Future Investigations

An important conclusion of this research is that it points out that future studies are needed to better understand the impact of parental DU or WA exposure on their children. This study raises

as many questions as we have endeavored to answer. Several areas of research that require immediate attention are:

1) Susceptibility of genomically unstable offspring to carcinogens
2) Maternal exposure and offspring genomic instability
3) Offspring carcinogenesis
4) Transplacental carcinogenesis

Appendices

Table 1	Litter Characteristics – Offspring Survival at Birth
Table 2	Litter Characteristics – Offspring Survival at Weaning
Table 3	Offspring Genotyping Analysis
Table 4	Mutation Frequency in Unexposed Offspring
Table 5	Mutation Frequency in Implanted Males
Table 6	Sperm DNA Damage in Implanted Males
Figure 1	Testes Metal (Uranium) Concentration
Figure 2	Testes Metal (Tungsten, nickel, cobalt) Concentration: One Month
Figure 3	Testes Metal (Tungsten, nickel, cobalt) Concentration: Four Months
Figure 4	Testes Metal (Tungsten, nickel, cobalt) Concentration: Seven Months

Table 1. Litter Characteristics:
Average Number of Offspring at Birth

Time Point	Control	Tantalum	DU- Low	DU- High	WA- Low	WA- High	Neutron
One Month	6.7 +/ 0.5	6.9 +/ 0.5	5.3 +/ 0.4	5.1 +/ 0.4	8.4 +/ 0.7	7.3 +/ 0.6	none
Four Months	6.5 +/ 0.5	5.2 +/ 0.4	5.7 +/ 0.5	5.0 +/ 0.4	5.4 +/ 0.5	5.5 +/ 0.6	
Seven Months	5.7 +/ 0.5	5.1 +/ 0.4	4.1 +/ 0.3	3.9 +/ 0.4	6.1 +/ 0.5	6.2 +/ 0.5	
68 Days							5.2 +/ 0.5

Table 2. Litter Characteristics:
Average Number of Offspring at Weaning

Time Point	Control	Tantalum	DU- Low	DU- High	WA- Low	WA- High	Neutron
One Month	6.7 +/ 0.5 (100)	6.3 +/ 0.5 (91)	4.8 +/ 0.4 (91)	4.6 +/ 0.4 (90)	7.6 +/ 0.6 (90)	6.9 +/ 0.6 (95)	no data
Four Months	6.1 +/ 0.5 (94)	4.7 +/ 0.4 (90)	5.0 +/ 0.5 (88)	4.4 +/ 0.4 (88)	4.9 +/ 0.5 (91)	4.9 +/ 0.6 (88)	
Seven Months	5.4 +/ 0.5 (95)	4.6 +/ 0.4 (90)	3.6 +/ 0.3 (88)	3.0 +/ 0.4 (77)	4.4 +/ 0.5 (72)	4.5 +/ 0.5 (73)	
68 Days							4.6 +/ 0.5 (88)

Table 3:
Genotyping of Offspring for Transmission of *lacI* Gene

Pooled Data from All Groups

C57Bl x Big Blue F1 Offspring	Control and Tantalum	DU and WA
No. Screened for *lacI* gene	599	1337
No. lacI carriers (*lacI*+)	281	620
Percent *lacI* carriers	47.1	46.4

Table 4

Mutation Frequency in Offspring Bone Marrow

Time Point	Control	Tantalum	DU- Low	DU- High	WA- Low	WA- High	Neutron
One Month	2.12 +/ 0.54	2.19 +/ 0.59	2.76 +/ 0.49	3.02 +/ 0.63	2.43 +/ 0.76	2.73 +/ 0.44	no data
Four Months	2.30 +/ 0.14	2.21 +/ 0.32	3.74 +/ 0.43	3.95 +/ 0.39	3.21 +/ 0.89	3.72 +/ 0.88	
Seven Months	2.13 +/ 0.44	2.04 +/ 0.42	3.86 +/ 0.38	4.87 +/ 0.45	3.51 +/ 0.68	3.88 +/ 0.61	
68 Days	-	-	-	-	-	-	4.2 +/= .66

Table 5

Mutation Frequency in Implanted Males

Testes Tissue

Time Point	Control	Tantalum	DU- Low	DU- High	WA- Low	WA- High	Neutron
One Month	1.23 +/ 0.35	1.37 +/ 0.79	6.57 +/ 2.27	12.96 +/ 3.07	4.65 +/ 3.51	5.29 +/ 4.64	28.78 +/ 7.12
Four Months	1.32 +/ 0.36	1.05 +/ 0.91	33.58 +/ 5.07	47.25 +/ 6.37	24.8 +/ 4.87	34.52 +/ 15.44	
Seven Months	1.87 +/0.46	1.44 +/ 0.867	46.45 +/ 7.81	70.92 +/ 9.24	44.42 +/ 8.31	51.02 +/ 8.77	

Mutation Frequency in Implanted Males

Kidney Tissue

Time Point	Control	Tantalum	DU- Low	DU- High	WA- Low	WA- High	Neutron
One Month	1.45 +/ 0.97	1.67 +/ 0.66	16.14 +/ 3.55	27.28 +/ 4.85	12.18 +/ 6.49	22.58 +/ 6.64	21.7 +/ 14.78
Four Months	1.85 +/ 0.91	1.93 +/ 1.10	37.76 +/ 3.69	60.46 +/ 10.43	29.1 +/ 8.06	53.46 +/ 12.51	
Seven Months	4.72 +/ 1.28	2.27 +/ 1.75	75.48 +/ 6.34	100.77 +/ 7.56	69.06 +/ 12.04	91.88 +/ 13.52	

Mutation Frequency in Implanted Males

Liver Tissue

Time Point	Control	Tantalum	DU- Low	DU- High	WA- Low	WA- High	Neutron
One Month	0.96 +/ 0.95	2.91 +/ 1.23	22.34 +/ 2.69	24.88 +/ 5.62	18.88 +/ 6.92	16.52 +/ 8.9	14.78 +/ 3.23
Four Months	1.09 +/ 0.92	3.93 +/ 1.04	51.70 +/ 4.35	64.54 +/ 11.09	52.74 +/ 6.51	63.76 +/ 13.77	
Seven Months	5.18 +/ 2.59	3.41 +/ 2.03	89.06 +/ 14.19	102.79 +/ 17.17	85.34 +/ 13.90	97.12 +/ 14.60	

Table 6

Sperm DNA Damage in Implanted Males
Tail length (um)

Time Point	Control	Tantalum	DU- Low	DU- High	WA- Low	WA- High	Neutron
One Month	38.58 +/ 1.67	39.1 +/ 3.50	42.58 +/ 1.35	47. 04 +/ 2.39	41.94 +/ 1.91	42.42 +/ 3.76	68.88 +/ 1.97
Four Months	38.24 +/ 2.03	39.84 +/ 3.76	51.74 +/ 2.76	60.82 +/ 1.11	49. 12 +/ 2.92	60.48 +/ 0.97	
Seven Months	39.66 +/ 1.22	40.84 +/ 5.06	64.00 +/ 2.78	70.92 +/ 4.96	62.76 +/ 3.31	59.54 +/ 3.48	

Sperm DNA Damage in Implanted Males
Tail DNA Percent

Time Point	Control	Tantalum	DU- Low	DU- High	WA- Low	WA- High	Neutron
One Month	1.45 +/ 0.97	1.67 +/ 0.66	16.14 +/ 3.55	27.28 +/ 4.85	12.18 +/ 6.49	22.58 +/ 6.64	21.7 +/ 14.78
Four Months	1.85 +/ 0.91	1.93 +/ 1.10	37.76 +/ 3.69	60.46 +/ 10.43	29.1 +/ 8.06	53.46 +/ 12.51	
Seven Months	4.72 +/ 1.28	2.27 +/ 1.75	75.48 +/ 6.34	100.77 +/ 7.56	69.06 +/ 12.04	91.88 +/ 13.52	

Sperm DNA Damage in Implanted Males
Tail Moment

Time Point	Control	Tantalum	DU- Low	DU- High	WA- Low	WA- High	Neutron
One Month	0.96 +/ 0.95	2.91 +/ 1.23	22.34 +/ 2.69	24.88 +/ 5.62	18.88 +/ 6.92	16.52 +/ 8.9	14.78 +/ 3.23
Four Months	1.09 +/ 0.92	3.93 +/ 1.04	51.70 +/ 4.35	64.54 +/ 11.09	52.74 +/ 6.51	63.76 +/ 13.77	
Seven Months	5.18 +/ 2.59	3.41 +/ 2.03	89.06 +/ 14.19	102.79 +/ 17.17	85.34 +/ 13.90	97.12 +/ 14.60	

Figure 1

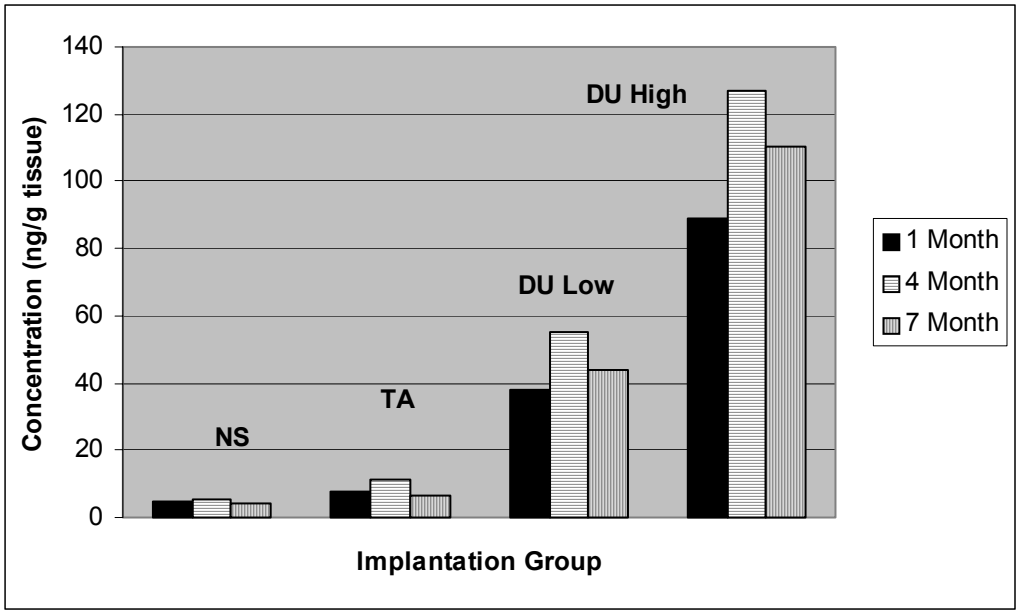

Figure 1. Testes Metal (Uranium) Concentrations: Months 1, 4, and 7. Data are the mean from 3 independent samples.

Figure 2

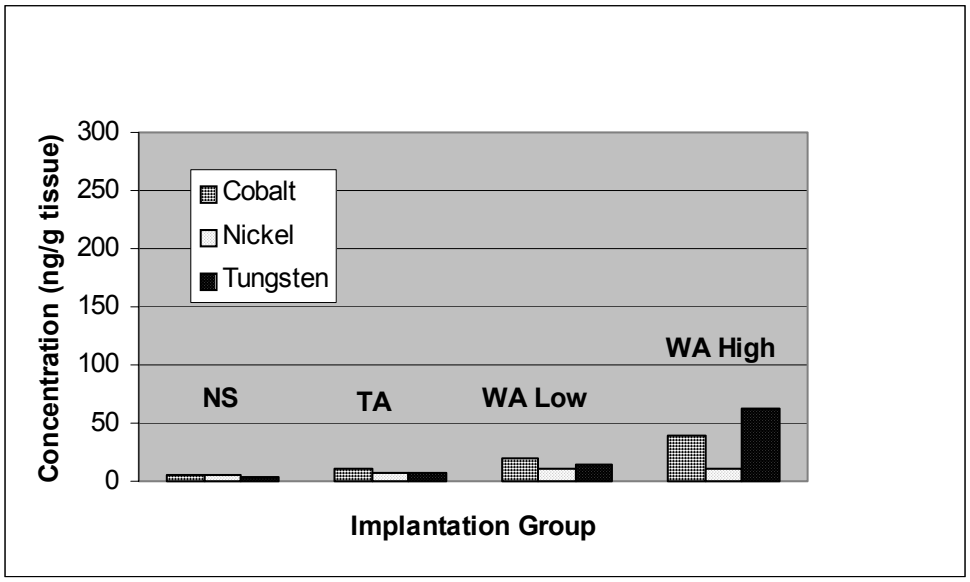

Figure 2. Testes Metal (Cobalt, Nickel, Tungsten) Concentrations: Month 1. Data are the mean from 3 independent samples.

Figure 3

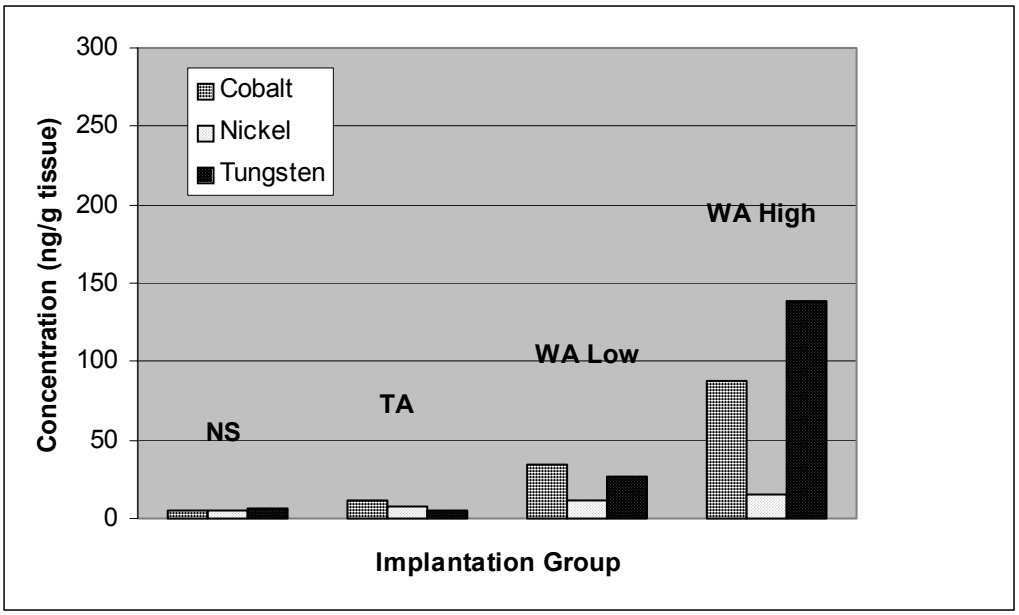

Figure 3. Testes Metal (Cobalt, Nickel, Tungsten) Concentrations: Month 4. Data are the mean from 3 to 5 independent samples.

Figure 4

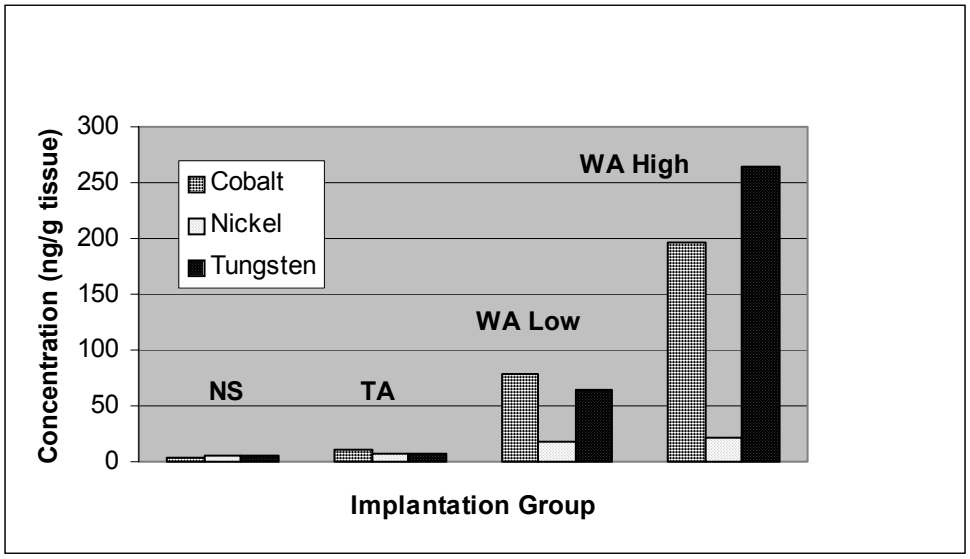

Figure 4. Testes Metal (Cobalt, Nickel, Tungsten) Concentrations: Month 7. Data are the mean from 3 to 5 independent samples.